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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12M 1/34 — A1

(11) International Publication Number: WO 91/01365

(43) International Publication Date: 7 February 1991 (07.02.91)

(21) International Application Number: PCT/US90/04025

(22) International Filing Date: 18 July 1990 (18.07.90)

(30) Priority data:
382,483 — 18 July 1989 (18.07.89) — US

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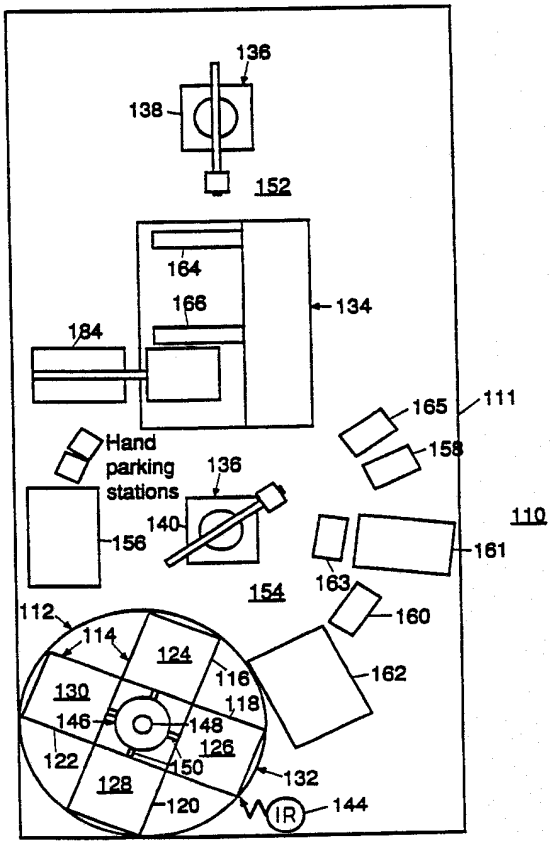
(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), SU.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: AUTOMATED LABORATORY APPARATUS

(57) Abstract

Laboratory apparatus (110) comprises an incubator (112) formed with a housing (114) substantially enclosing an interior space. The incubator maintains in the interior space prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells. A liquid handling station (134) is provided, and a robot (136) transfers to the liquid handling station (134) cells from the incubator (112) and chemicals that when mixed with the cells to form a mixture that is then stored under the prescribed conditions produce a detectable change in the cells. The liquid handling station (134) includes nozzles for mixing the chemicals with the cells to form a mixture, and the robot transfers the mixture to the incubator (112) for incubation therein. The robot transfers the cells after their incubation to a luminometer (162) that produces a detection output signal in response to the detectable change in the cells.



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AUTOMATED LABORATORY APPARATUS

Background of the Invention

This invention relates to laboratory apparatus and more particularly to novel and highly-effective automated laboratory apparatus that enables inexpensive, reliable, and high-speed testing of the effects of a very large number of chemicals on selected substances such as collections of living cells. The invention relates also to laboratory dishes and more particularly to a novel and highly-effective laboratory dish that is tissue-culture compatible and specially adapted for cooperation with automated apparatus to facilitate data collection.

The testing of the effects of various chemicals on selected samples such as collections of living cells is known. It may be necessary to determine, for example, which of a large class of chemicals have any effect on the growth and reproduction of, or production of a particular protein by, a collection of cells of a given strain and to rank the effects produced. This can of course be done "manually" by preparing collections of cells, adding solutions of the chemicals to be investigated in various dilutions to the respective cell collections, and observing the results.

In practice, such a manual procedure is not merely tedious but impractical in view of the astronomical number of combinations of cell strains and chemicals that one would like to test. Moreover, the entire procedure requires

multiple handling of the cells and chemicals, since the cells, which must be kept alive for an extended period in order to maximize the information available from the testing, need to be incubated under closely controlled conditions, and the chemicals may need to be dissolved and prepared in multiple dilutions immediately before the testing begins.

Very sophisticated laboratory apparatus facilitating a high degree of automation is of course known to those skilled in the art and available for testing, material handling, data processing, and general laboratory purposes. For example, a robotic system with interchangeable hands is disclosed in a U.S. patent to Hutchins et al. No. 4,488,241. This patent is incorporated herein by reference pursuant to MPEP 608.01(b)(B). Zymark Corporation of Hopkinton, Massachusetts, markets a robotic system model Zymate II that incorporates certain features disclosed in the patent. Similarly, Tecan AG of Landhaus Holgass, Switzerland, markets a liquid handling station model RSP 5052 that can be programmed to prepare various dilutions of chemicals for testing purposes. Other sophisticated equipment that is well known to those skilled in the art is available commercially as shown in the following table:

TABLE

Supplier	Location	Model	Device
Dynatech	Chantilly, VA	ML 1000	Luminometer
Bio-Tek	Winooski, VT	EL 403	Plate Washer
Mettler	Hightstown, NJ	AE 240	Balance
Dynatech			4-Position microplate shaker
Perkin Elmer Cetus	Norwalk, CT	Pro/Pette	Fibronectin coater and cell plater

In addition, a general purpose digital computer can be used to collect and process data from the luminometer or other means for producing a signal, as those skilled in the art will readily understand.

All of the commercially available devices disclosed above are suitable for use in accordance with the present invention, and of course other systems available now or to be made available in the future can be substituted for those identified above so long as they are capable of performing the functions required by the invention as outlined below.

Individually the various pieces of sophisticated laboratory equipment known to those skilled in the art and commercially available make significant contributions to the efficiency and repeatability of various laboratory procedures. Collectively, however, in conventional practice they amount to no more than the sum of the individual pieces of equipment, since the interface between the various pieces of equipment is not fully automated; that is, each piece of equipment performs only its conventional function, and laboratory personnel must still tend the equipment to complete the necessary interfacing.

Laboratory dishes are of course known to those skilled in the art and are in fact seemingly ubiquitous in the laboratory. A typical laboratory dish is formed with a plurality of wells for holding separate samples of solutions, suspensions, etc., so that the solutions, suspensions, etc., or components thereof, or their reactions with other materials such as collections of cells can be examined or tested separately. A popular and useful

laboratory dish is made of plastic and given a special treatment to make it hydrophilic and compatible with living cells and has 96 wells arranged in an 8 x 12 rectangular array.

Conventional laboratory dishes that are tissue-culture compatible isolate the contents of each well from the contents of all other wells but do not provide signal isolation as explained below and therefore do not lend themselves to the reliable, high-speed collection of certain kinds of data. For example, a light signal produced in a first well may travel through the dish, which is translucent, and enter a second well, so that a luminometer juxtaposed to the second well detects light generated not only there but also in the first well. This leads to confusion about the properties of the materials contained in the two wells.

Summary of the Invention

An object of the invention is to remedy the deficiencies of the prior art noted above and in particular to provide automated laboratory apparatus that enables inexpensive, reliable, and high-speed testing of the effects of a very large number of chemicals on selected samples such as collections of living cells.

Another object of the invention is to provide a novel and highly-effective laboratory dish that is specially adapted for cooperation with automated apparatus to facilitate reliable and high-speed data collection.

Other objects of the invention are:

To provide laboratory apparatus comprising a number of automated components and means whereby the interface between any two cooperating components is automated;

To provide laboratory apparatus that can run completely unattended for extended periods, thereby freeing laboratory personnel to devote more time to the performance of creative and other high-level functions than would otherwise be possible;

To provide automated apparatus that provides greater reliability and repeatability of various laboratory procedures including the preparation of materials for testing, the performance of testing, and the collection of data;

To provide a laboratory plate or dish that is tissue-culture compatible, has a plurality of wells for holding separate cell samples, and is adapted to provide signal isolation between wells, whereby a signal generated in one well is prevented from penetrating into another well; and

To provide a laboratory plate or dish that is tissue-culture compatible, has a plurality of wells for holding separate cell samples, and is adapted to provide signal isolation between wells, whereby signals generated within each well can be detected separately without contamination by signals generated within other wells.

The foregoing and other objects of the invention are attained in accordance with a first aspect thereof by the provision of laboratory apparatus comprising: incubator means formed with housing means substantially enclosing an interior space, the incubator means maintaining in the interior space prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells; handling means; detection means; and robotic means; the robotic means transferring to the handling means cells from the incubator means and chemicals that when mixed with the cells to form a mixture that is then stored under the prescribed conditions produce a detectable change in the cells; the handling means comprising mixing means for mixing the chemicals with the cells to form a mixture; the robotic means transferring the mixture to the incubator means for incubation therein; and the detection means receiving the mixture after the incubation and producing a detection signal in response to said detectable change.

In accordance with the invention, the robotic means may interface between the incubator means and the handling means or between the incubator means and the detection means. Preferably, however, it interfaces both between the incubator means and the handling means and between the incubator means the the detection means.

In accordance with an independent aspect of the invention there is provided a testing method comprising the steps of establishing within an incubator prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells; storing within the incubator cells from the predetermined collection; robotically transferring from the incubator to a liquid handling station cells from the incubator and chemicals that when mixed with the cells to form a mixture that is then stored under the prescribed conditions produce a detectable change in the cells; mixing at the liquid handling station the chemicals with the cells to form a mixture; robotically transferring the mixture to the incubator for incubation therein; and producing in a detector a detection signal in response to said detectable change.

As in the case of the apparatus of the invention, in accordance with the method of the invention the transfers between the incubator and the liquid handling station or between the incubator and the detector are effected robotically; and preferably transfers both between the incubator and the liquid handling station and between the incubator and the detector are effected robotically.

In accordance with another independent aspect of the invention there is provided a laboratory plate formed with a

plurality of wells for respectively containing substances that emit a detectable signal, the dish being tissue-culture compatible and formed in such a manner as to block the transmission of the signal from each of the wells to others thereof, thereby enabling detection of the signal from each of the cells separately.

The foregoing and other objects, features, and advantages of the invention may be better understood from a consideration of the following detailed description of the preferred embodiments thereof, in conjunction with the appended figures of the drawing.

Brief Description of the Figures

Fig. 1 is a plan view of the overall layout of apparatus constructed in accordance with the invention.

Fig. 2 is a perspective view of a liquid handling station employed in accordance with the invention and forming a part of the apparatus of Fig. 1.

Fig. 3 is a schematic flow chart giving an overall view of the process of the invention.

Fig. 4 is a plan view of a master plate and a 32-sample vial rack contained on a master plate tray and employed in the apparatus of Fig. 1.

Fig. 5 is a plan view of six cell plates contained on a cell plate tray and employed in the apparatus of Fig. 1.

Fig. 6 is a detailed plan view showing the layout of the master plate of Fig. 4.

Fig. 7 is a detailed plan view showing the layout of a typical one of the six cell plates of Fig. 5.

Fig. 8 is a flow chart of a main program for controlling a first arm of the liquid handling station of Fig. 2.

Fig. 9 is a flow chart of a subroutine employed in the program of Fig. 8.

Fig. 10 is a flow chart of a main program for controlling a second arm of the liquid handling station of Fig. 2.

Fig. 11 is a flow chart of a subroutine employed in the program of Fig. 10.

Detailed Description of the Invention

Fig. 1 shows laboratory apparatus 110 constructed in accordance with the invention. It can be mounted for convenience on any suitable support such as a table 111, and it comprises incubator means 112 including housing means enclosing an interior space (which may be subdivided as indicated below) in which conditions appropriate to survival and reproduction of at least one predetermined collection of living cells are maintained. The housing 114 may comprise for example four separate compartments 116, 118, 120 and 122 each enclosing an interior space respectively represented schematically at 124, 126, 128 and 130. Each compartment is provided with shelves or sets of tray guides so that each compartment is adapted to hold ten trays each of which supports six laboratory cell plates or dishes. The trays are arranged one above another in each compartment. The prescribed conditions maintained within the incubator compartments 116, 118, 120 and 122 in accordance with the invention may include for example a temperature of substantially 37°C, a relative humidity of nearly 100%, and a 5% concentration by volume of carbon dioxide (CO_2). The incubator 112 further comprises a turntable 132 whereby the housing 114 can be rotated so as to present successively the compartments 116, 118, 120 and 122 to the work space of a robot discussed below.

The laboratory apparatus 110 further comprises handling means such as a liquid handling station 134 and robotic means 136 which for convenience is divided into a first robot 138 and a second robot 140. The robotic apparatus 136, and particularly the robot 140 thereof, transfers cells

or another sample to be exposed to various chemicals for testing purposes from the incubator 112 to the liquid handling station 134. The robotic apparatus 136 and particularly the first robot 138 thereof transfers to the liquid handling station 134 chemicals that when mixed with the cells to form a mixture that is then stored under the prescribed incubator conditions described above produce a detectable change in the cells.

Means is also provided for detecting the detectable change in the cells and for producing a detection output signal in response thereto. For example, where the detectable change in the cells involves the production of photons, the detection means preferably comprises a luminometer 162 (Fig. 1). After incubation of the cells on the cell plates within the incubator 112 for a period of preferably about six hours, the robotic apparatus 136 and particularly the second robot 140 thereof transfers the cells to the luminometer 162. The luminometer includes a photomultiplier plus means whereby a relative scan is established between the photomultiplier and the various wells in the cell plates. The cell plates are of special construction as described below, whereby a signal generated within each well can be detected separately without contamination by signals generated within other wells.

As Fig. 2 shows, the liquid handling station comprises mixing means 142 for mixing the chemicals with the cells as explained in detail below to form the mixture which the second robot 140 of Fig. 1 then transfers to the incubator 112 for incubation therein. In order to maintain in the incubator 112 a controlled flow of warm, humid air containing the desired concentration of CO_2, a coupling 146

is provided communicating with the interior space 124, 126, 128 and 130 for blowing humidified air into the interior space through an entrance port 148 and from the interior space through an exit port 150, the entrance and exit ports being arranged coaxially with respect to each other and with the turntable 132. The incubator turntable 132 is preferably made of metal or another good conductor of heat and irradiated from below by an infrared lamp 144 or other heating source to assist in maintaining the required temperature of substantially 37°C.

The robotic apparatus 136 has a defined work space 152, 154, the work space 152 being proper to the first robot 138 and the work space 154 being proper to the second robot 140. As indicated above, the housing 114 of the incubator 112 is formed with the four compartments 116, 118, 120 and 122, and the turntable 132 rotates the housing so that different ones of the compartments are selectively moved into the work space 154 of the second robot 140. The robot 140 is thus able to withdraw objects from and insert objects into a selected one of the compartments 116, 118, 120, 122 at appropriate times as described below.

Fig. 3 presents an overall view of the steps performed in accordance with the invention by the apparatus of Fig. 1. Two different preparatory operations are performed off-line. To prepare the cells, 96-well microtiter dishes or plates described in detail below are given a tissue culture pretreatment and sterilized. Typically such pretreatment and sterilization is done by the supplier of the dishes. One such supplier is Dynatech, identified above. Then fibronectin coating of the plates is performed. This can be done using a Cetus Pro/pette cell plater. Cells containing

target substances are then plated onto the 96-well microtiter plates along with a nutrient solution. The Cetus Pro/pette will also perform this function. Six plates thus loaded with cells are placed on a tray, and the tray is manually transferred to the incubator 112. Periodically, for example every 16 hours, the incubator 112 is fully loaded in this way so that it contains about 20,000 cells per well (enough to produce a signal that is readily detectable) x 96 wells per cell plate, x 6 cell plates per tray, x 10 trays per compartment, x 4 compartments per incubator. The processing rate of the apparatus (on average less than 2.5 seconds per reading, including waiting time for changing cell plates, etc.) is such that the apparatus loaded in this way will run completely unattended between loadings and will produce in that interval readings corresponding to about 23,000 wells.

The other off-line preparation relates to the chemicals employed and includes storage, data file entry and vial coding. Optionally a robotic bar coder may be employed to facilitate this task.

At this point the robotic operation preferably begins. The operation may be briefly summarized as follows: The liquid handling station loads an identical set of chemicals onto each of six cell plates supported on a cell plate tray. Each cell plate contains cells of a different cell line having a particular characteristic. The cells for each cell plate are contained in 96 separate wells. Thus six different cell lines can be screened simultaneously. The first robot 138 loads onto the liquid handling station 134 a set of 32 vials and an empty 96-well master plate supported on a master plate tray. The first arm 164 (Fig. 2) of the

liquid handling station 134 uses the master plate 170 to prepare three dilutions as explained below for every chemical. The second arm 166 of the liquid handling station 134 then transfers the chemicals from the master plate to the six cell plates located on a cell plate tray. The second robot 140 then transfers the cell plate tray to the incubator 112. After incubation the plates are processed individually in the luminometer 162 of Fig. 1.

In somewhat more detail, the robotic operation proceeds as follows (Fig. 3): The operation begins with a weighing (for example on a Mettler balance) of chemicals into vials and dissolution in a suitable solvent such as dimethylsulfoxide (DMSO) or a cage molecule sold under the trademark Molecusol. The vials containing the weighed and dissolved chemicals are transferred by the first robot 138 to the liquid handling station 134.

The second robot 140 then withdraws a cell plate tray supporting six cell plates each containing 96 wells arranged in an 8 x 12 array (Fig. 5) from the incubator 112 (Fig. 1), places the cell plate tray onto a tray location station 156 shown in Fig. 1, removes the lids from each plate separately and transfers them to a lid parking station 158, and then transfers the tray with the six uncovered plates to the liquid handling station 134. The first robot 138 has meanwhile transferred a tray with the chemical vials and master plate to the liquid handling station 134 as indicated above, and the liquid handling station 134 adds the chemicals to the cells in the plates. The second robot 140 then transfers the tray supporting the plates to the tray locating station 156, replaces on the plates the lids that have been temporarily stored at the lid parking station 158 and then transfers the tray with the plates to the incubator

112.

As Fig. 3 indicates, the plates containing the cells thus treated are then incubated in the incubator 112, typically for six hours in a humidified atmosphere maintained at 37°C and containing a 5% concentration by volume of CO_2, as explained above.

The second robot 140 then unloads the tray containing the six cell plates to the tray locating station 156, removes the lids from the cell plates, discharges the lids into a chute 160, and moves the tray containing the cell plates to the plate washer 161. The plate washer 161 washes away the chemicals and the robot 140 moves the plate to the reagent addition station 165, which adds one or more additional chemicals if needed to facilitate the generation of a signal representative of the changes effected in the cells by virtue of the addition of the first group of chemicals. For example the second chemical or set of chemicals may include a detergent to rupture the cell membranes so that the contents of the cells are disgorged and a chemical that reacts with the contents to generate a detectable signal.

The second robot 140 then transfers the plates to a detection device selected in accordance with the nature of the signal to be generated. For example, if the signal to be generated is a light signal, the second robot 140 transfers the plates into the luminometer 162. A plate turntable 163 is provided upon which the second robot 140 places a plate to enable reorientation of the plate so that it can be properly accommodated in the various pieces of equipment (luminometer 162, etc.). The luminometer 162 measures a bioluminescent signal, which is supplied to a computer for data processing. After the scanning of each

cell plate by the luminometer 162 is completed, the second robot 140 removes that plate from the luminometer 162 and discards it into the chute 160.

The process outlined above is set out in still greater detail below in connection with Figs. 4-7. Fig. 4 shows a master plate tray 168 holding a 96-well master plate 170 and a 32-sample vial rack 172. See also Fig. 2. The 32 vials formed in the 32-sample vial rack 172 and the samples (chemicals) respectively contained in those vials are arranged from 1 to 32 in the order shown in Fig. 4.

Both the first and the second arms 164 and 166 of the liquid handling station 134 are independently movable in three dimensions X, Y and Z. The arm 164 can approach any of the vials in the 32-sample vial rack and any of the wells in the 96-well master plate. The first arm 164 is equipped with two nozzles 164a and 164b that are close enough to each other that, with the first arm 164 in a given position, the nozzles 164a and 164b can cooperate with the same well of the master plate 170 or vial of the 32-sample vial rack 172. The nozzles 164a and 164b are separately controllable to aspirate or dispense. The nozzles are made to enter the first vial of the 32-sample vial rack, and one nozzle 164a withdraws a sample.

The arm 164 then moves so that the nozzles 164a and 164b are juxtaposed to the well of the master plate 170 labeled sample 1, first dilution (see Fig. 6). The first sample aspirated from the 32-sample vial rack is then dispensed into the given well by the one nozzle 164a, and a diluent is dispensed by the other nozzle 164b to form the first dilution of the first sample. The arm 164 then moves the nozzles 164a and 164b to the well labeled sample 1, second

dilution, and the nozzle 164b dispenses only diluent. Then the arm 164 moves the nozzles 164a and 164b to the well labeled sample 1, third dilution, and the nozzle 164b again dispenses only diluent.

Sample 1 contained in vial 1 of the 32-sample vial rack of Fig. 4 is thus deposited by the first arm 164 of the liquid handling station 134 in a first dilution as shown by heavy shading in the lower right well of the master plate shown in Fig. 6, and at this point diluent only is in the sample 1, second and third dilution wells, which are respectively indicated by progressively lighter shading.

Similarly, sample 2 contained in vial 2 of the 32-sample vial rack 172 shown in Fig. 4 is distributed with diluent into the sample 2, first dilution, well shown to the left of the well holding sample 1, first dilution in Fig. 6, and only diluent is added at this point to wells labeled sample 2, second and third dilutions. In the same way, sample 3 of the 32-sample vial rack 172 (Fig. 4) is distributed in a first dilution to the left of sample 2, first dilution, in Fig. 6; sample 4, completing the left column of vials in Fig. 4, is distributed in a first dilution to the left of sample 3, first dilution, in Fig. 6; and only diluent is at this point added to the wells of Fig. 6 labeled samples 3 and 4, second dilution, and samples 3 and 4, third dilution.

With four wells in the right half of the bottom row of Fig. 6 thus respectively loaded with four different samples in a first dilution, and with diluent in the remaining eight wells as mentioned above, the second arm 166 of the liquid handling station 134 then moves to a first position wherein its four nozzles 174, 176, 178 and 180 (Fig. 2) respectively enter the wells containing sample 1, first dilution; sample

2, first dilution; sample 3, first dilution; and sample 4, first dilution. The nozzles thoroughly mix the contents of these wells by aspirating and dispensing the contents a plurality of times, for example three times. An aliquot portion of the thoroughly mixed contents in the first dilution is then transferred by the second arm 166 to wells labeled samples 1-4, second dilution, and dispensed through the nozzles 174-180. The second arm 166 of the liquid handling station 134 then moves the nozzles 174-180 to the cell plate of Fig. 7 and dispenses the first dilution through the respective nozzles 174-180 into 16 wells labeled sample 1, first dilution; sample 2, first dilution; sample 3, first dilution; and sample 4, first dilution. Each of the nozzles 174-180 aspirates and dispenses a different sample.

The cell plate of Fig. 7 is representative of the six cell plates of Fig. 5, and the second arm 166 dispenses in the same manner into 16 wells in each of the six cell plates.

The second arm 166 then moves the nozzles 174-180 to respective positions corresponding to the wells of the master plate containing sample 1, second dilution; sample 2, second dilution; sample 3, second dilution; and sample 4 second dilution. It will be recalled that up to now these wells contain only diluent. The nozzles 174-180 then respectively dispense the first dilution of samples 1-4 into these wells and again cause thorough mixing by repeated aspiration and ejection. This forms the second dilution.

The second arm 166 aspirates an aliquot portion of the thoroughly mixed second dilution, moves to the cell plate wells of Fig. 7 identified as containing second dilutions of the first four samples, dispenses into those 16 wells, and

then dispenses in the same manner into a corresponding 16 wells in each of the other five cell plates in Fig. 5. Thus each of the six cell plates shown in Fig. 5 is loaded with four replicates of samples 1-4, second dilution, as illustrated in Fig. 7.

The second arm 166 then moves back to the master plate in Fig. 6, dispenses the second dilutions of samples 1-4 through nozzles 174-180, respectively, into the third dilution wells of samples 1-4, so that it mixes with diluent already there, and thoroughly mixes by aspiration and ejection to form the third dilution, which it then distributes to the 16 wells of the cell plate of Fig. 7 that are identified as containing the third dilution of samples 1-4. It then dispenses in the same manner into the other five cell plates.

The process described above is repeated until each cell plate is filled as indicated in Fig. 7, whereby each of eight samples is represented in three different dilutions, each dilution of each sample being replicated four times on each of six cell plates. The amounts of diluent are selected relative to the amounts of the samples (and of the nutrient solution originally added) so that the dilutions differ from one to the next by a factor of, say, 10, in order to determine the effects of the samples (chemicals) on the cells over a wide range of concentrations. The reason for the replications of each concentration in the cell plates is to provide more meaningful statistical data than would be obtainable if each dilution of each sample occurred only once.

The process described above is essentially complete but for the sake of simplicity omits certain steps including rinsing

steps that are performed in order to prevent unintended cross-mixing of chemicals and shaking steps that are performed to ensure homogeneity.

Figs. 8-11 are flow charts showing the manner in which the first arm 164 and second arm 166 of the liquid handling station 134 are controlled and disclosing also the rinsing and shaking steps.

In Fig. 8, the main program for the first arm 164 is initialized at step 186. The program then moves to the load machine configuration at step 188. At step 190, the program requests the 32-sample vial rack 172 and master plate 170 contained on the master plate tray 168 (Fig. 4). It then waits at step 192 for a go signal from the robotic apparatus 136. The go signal is generated by the robotic apparatus 136 as programmed by its supplier. When the go signal is received from the robotic apparatus 136, the request signal generated at step 190 is canceled at step 194. The 32 samples are then processed at step 196 as shown in the subroutine of Fig. 9 discussed below. At step 198 the program waits for the second arm, which performs steps discussed below in connection with Figs. 10 and 11. If the program stop signal is detected at step 200, the program terminates at step 202. Otherwise, it loops back to step 190 and repeats steps 192-200, including the subroutine referred to at step 196, until the program stop signal is detected.

The subroutine referred to at step 196 is shown in detail in Fig. 9. The subroutine begins at step 204, at which the first arm 164 gets a sample from the 32-sample vial rack 172 and transfers it to the master plate 170 in the manner outlined above. At step 206, the diluent is added and the

mixing is done. At step 208, the diluent is loaded into two more wells as specified above. If four samples have not yet been prepared in this manner as determined at step 210, the program moves to step 212, and if four samples are ready as determined at step 210, the program first sets the go signal for the second arm 166 at step 214, thereby putting the second arm 166 into play as outlined below, and then proceeds to step 212. At step 212, the program sets the stop signal for the arm 166 and causes the arm 164 to rinse the nozzles 164a and 164b at the wash station 213 of Fig. 2. If 32 samples have been processed as determined at step 216, the program returns to the main program of Fig. 8 as indicated at step 218. If 32 samples have not yet been processed as determined at step 216, the program loops back to step 204 and repeats.

Fig. 10 shows the main program for the second arm 166 of the liquid handling station 134. The second arm 166 is initialized at step 220. At step 222, the routine causes the apparatus to process the samples and load the cell plates. This is done in accordance with the subroutine of Fig. 11 discussed below. If at step 224 the program stop signal is detected, the program terminates as indicated at 226. If the program stop signal is not detected, the program loops back to step 222 and repeats until the program stop signal is detected.

Fig. 11 shows step 222 of the master program of Fig. 10 in detail. The program enters the subroutine of Fig. 11 at step 228, which causes the four nozzles 174-180 of the second arm 166 to mix four samples in the master plate and pick an aliquot portion thereof. If at step 230 it is determined that three dilutions have been done, the program proceeds to step 232. If at step 230 it is determined that

three dilutions have not been done, the program proceeds first to step 234 in which it causes the transfer of an aliquot portion into the next well and then to step 232. At step 232, the program causes the nozzles 174-180 of the second arm 166 to dispense diluted samples into the cell plates. At step 236, the program causes the nozzles 174-180 to move to the wash station 237 (Fig. 2). At step 238, the program causes the plate shaker 184 of Fig. 1 to switch on for fifteen seconds, and the nozzles 174-180 are washed at station 237. If at step 240 it is determined that the cell plates are not all filled, the subroutine of Fig. 11 loops back to step 228 and repeats until it is determined at step 240 that all of the cell plates have been filled. When at step 240 it is determined that the cell plates have all been filled, the program causes the apparatus to request an exchange of cell plates at step 242. At step 244, the second arm 166 waits for the go signal from the robotic apparatus 136. If it is determined at step 246 that 32 samples have not yet been processed, the program loops back to step 228 and repeats. When it is determined at step 246 that 32 samples have been processed, the program returns to the main program of Fig. 10 as indicated at step 248.

Conventional cell plates that are tissue-culture compatible are made of a translucent plastic. When a bioluminescent signal is developed in a well of such a conventional plate, the signal can pass through the translucent plastic into an adjacent well. Accordingly, a luminometer of which the photomultiplier is in the process of scanning the adjacent well may detect not only light signals generated in the adjacent well but also light signals generated in the first-named well and other nearby wells. Since the signals generated in the respective wells are not properly isolated, there is confusion regarding the nature of the materials

contained in the wells. This problem is avoided in accordance with the present invention by designing the plate for signal isolation. This can be done for example by making the plate of an opaque substance such as an opaque plastic suitable for fibronectin coating and cell plating. It is not necessary, however, that the entire plate be made of an opaque material. All that is required is that light signals from one well be prevented from penetrating into other wells. Accordingly, an opaque checkerboard grid can be employed to define for example square compartments that respectively surround each of the wells. Such grid extends from the top to the bottom of the dish and the dishes can otherwise be made of a conventional material including translucent or transparent plastic.

If the cells are capable of attaching to the bottoms of the wells formed in the dish, the wells can be flushed out from above preparatory to adding the second group of chemicals referred to above (e.g., a detergent to rupture the cell membranes and chemicals to react with the contents of the cells) without washing away the cells. However, if the cells are not capable of attaching to the bottoms of the wells and are in suspension, they cannot be washed in this manner without losing the cells. Accordingly, in accordance with an alternative embodiment of the invention, the bottoms of the wells are formed with a filter having a pore size smaller than the cells so that the wells can be washed by a liquid entering through the tops of the wells and exiting through the bottoms. The filters retain the cells in the respective wells during the washing. The filters may be made of the same plastic as the remainder of the plate or of a different substance.

Thus there is provided in accordance with the invention a

novel and highly-effective automated laboratory apparatus that enables inexpensive, reliable, and high-speed testing of the effects of a very large number of chemicals or selected substances such as collections of living cells. There is also provided in accordance with the invention a novel and highly-effective laboratory dish that is tissue-culture compatible and specially adapted for cooperation with automated apparatus to facilitate cell collection.

Many modifications of the preferred embodiments of the invention disclosed above will readily occur to those skilled in the art. For example, the number of dilutions per chemical, the number of cell plates per tray, the number of incubator compartments per incubator, and the number of discrete robots constituting the robotic apparatus 136 can be varied within wide limits, and the cell lines or other samples employed as well as the chemicals employed can be varied virtually without limit. Moreover, the robotic apparatus 136 can facilitate cooperation between the incubator and liquid handling station, between the incubator and luminometer, or between the incubator and both the liquid handling station and the luminometer. Also, the cell plates in accordance with the invention can be entirely opaque or can be partly translucent or transparent, but opaque to the extent necessary to prevent a signal such as light from traveling from one well to another. Many other modifications of the preferred embodiments of the invention disclosed herein will readily occur to those skilled in the art. The invention should therefore be construed as including all apparatus, methods and products that are within the scope of the appended claims.

What is claimed is:

1. Laboratory apparatus comprising:

incubator means formed with housing means substantially enclosing an interior space, said incubator means maintaining in said interior space prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells;

handling means;

detection means; and

robotic means;

said robotic means transferring to said handling means cells from said incubator means and chemicals that when mixed with said cells to form a mixture that is then stored under said prescribed conditions produce a detectable change in said cells;

said handling means comprising mixing means for mixing said chemicals with said cells to form a mixture;

said robotic means transferring said mixture to said incubator means for incubation therein; and

said detection means receiving said mixture after said incubation and producing a detection signal in response to said detectable change.

2. Laboratory apparatus comprising:

incubator means formed with housing means substantially enclosing an interior space, said incubator means maintaining in said interior space prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells;

handling means;

detection means; and

robotic means;

said handling means receiving cells from said incubator means and chemicals that when mixed with said cells to form a mixture that is then stored under said prescribed conditions produce a detectable change in said cells;

said handling means comprising mixing means for mixing said chemicals with said cells to form a mixture which is transferred to said incubator means for incubation therein;

said robotic means transferring said mixture after incubation in said incubation means to said detection means; and

said detection means producing a detection signal in response to said detectable change.

3. Laboratory apparatus comprising:

Incubator means formed with housing means substantially enclosing an interior space, said incubator means maintaining in said interior space prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells;

handling means;

detection means; and

robotic means;

said robotic means transferring to said handling means cells from said incubator means and chemicals that when mixed with said cells to form a mixture that is then stored under said prescribed conditions produce a detectable change in said cells;

said handling means comprising mixing means for mixing said chemicals with said cells to form a mixture;

said robotic means transferring said mixture to said incubator means for incubation therein;

said robotic means transferring said mixture after said incubation to said detection means; and

said detection means producing a detection signal in response to said detectable change.

4. Laboratory apparatus according to any of claims 1-3

wherein said incubator means comprises temperature control means for maintaining in said interior space a temperature of substantially 37°C.

5. Laboratory apparatus according to claim 4 wherein said temperature control means comprises infrared heating means for irradiating a portion of said incubator means.

6. Laboratory apparatus according to any of claims 1-3 wherein said incubator means comprises humidity control means for maintaining and interior space at a relative humidity of nearly 100%.

7. Laboratory apparatus according to claim 6 wherein said humidity control means comprises coupling means formed with entrance and exit ports communicating with said interior space for blowing humidified air into said interior space through said entrance port and from said interior space through said entrance port, said entrance and exit ports being arranged coaxially with respect to each other.

8. Laboratory apparatus according to any of claims 1-3 wherein said robotic means has a defined work space, said housing means is formed with a plurality of compartments, and said incubator means comprises turntable means mounting said housing means for rotation so that different ones of said compartments are selectively moved into said work space.

9. Laboratory apparatus according to any of claims 1-3 wherein said cells are contained in separate cell wells and said mixing means comprises nozzle means for preparing multiple dilutions of said chemicals and adding different ones of said multiple dilutions to different ones of said cell wells.

10. Laboratory apparatus according to claim 9 wherein said nozzle means comprises a first plurality of nozzles for respectively dispensing chemicals and diluent into master wells.

11. Laboratory apparatus according to claim 10 wherein said nozzles means further comprises a second plurality of nozzles for dispensing said dilutions into said cell wells.

12. Laboratory apparatus according to claim 10 wherein said detectable signal is light and said detection means comprises a luminometer including at least one photomultiplier.

13. Laboratory apparatus according to claim 12 wherein said mixture is contained in a plurality of discrete wells and said luminometer comprises scan means for producing a relative scan between said wells and said photomultiplier whereby said photomultiplier scans said wells successively.

14. A testing method comprising the steps of:

establishing within an incubator prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells;

storing within the incubator cells from said predetermined collection;

robotically transferring from the incubator to a liquid handling station cells from the incubator and chemicals that when mixed with the cells to form a mixture that is then stored under the prescribed conditions produce a detectable change in the cells;

mixing at the liquid handling station the chemicals with the cells to form a mixture;

robotically transferring the mixture to the incubator for incubation therein; and

producing in a detector a detection signal in response to said detectable change.

15. A testing method comprising the steps of:
establishing within an incubator prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells;

storing within the incubator cells from said predetermined collection;

transferring from the incubator to a liquid handling station cells from the incubator and chemicals that when mixed with the cells to form a mixture that is then stored under the prescribed conditions produce a detectable change in the cells;

mixing at the liquid handling station the chemicals with the cells to form a mixture;

transferring the mixture to the incubator for incubation therein;

robotically transferring the mixture after incubation in the incubator to a detector; and

producing in the detector a detection signal in response to said detectable change.

16. A testing method comprising the steps of;

establishing within an incubator prescribed conditions appropriate to survival and reproduction of at least one predetermined collection of living cells;

storing within the incubator cells from said predetermined collection;

robotically transferring from the incubator to a liquid handling station cells from the incubator and chemicals that when mixed with the cells to form a mixture that is then stored under the prescribed conditions produce a detectable change in the cells;

mixing at the liquid handling station the chemicals with the cells to form a mixture;

robotically transferring the mixture to the incubator for incubation therein;

robotically transferring the mixture after said incubation to a detector; and

producing in the detector a detection signal in response to said detectable change.

17. A laboratory plate formed with a plurality of wells for respectively containing samples that emit a detectable signal, said plate being tissue-culture compatible and formed in such a manner as to block the transmission of said signal from each of said wells to others thereof, thereby enabling detection of said signal from each of said wells separately.

18. A laboratory plate according to claim 17 wherein said signal is light and said plate is constructed of a material that is opaque in directions extending between said wells.

19. A laboratory plate according to claim 17 wherein said signal is light and said plate is constructed of a material that is opaque.

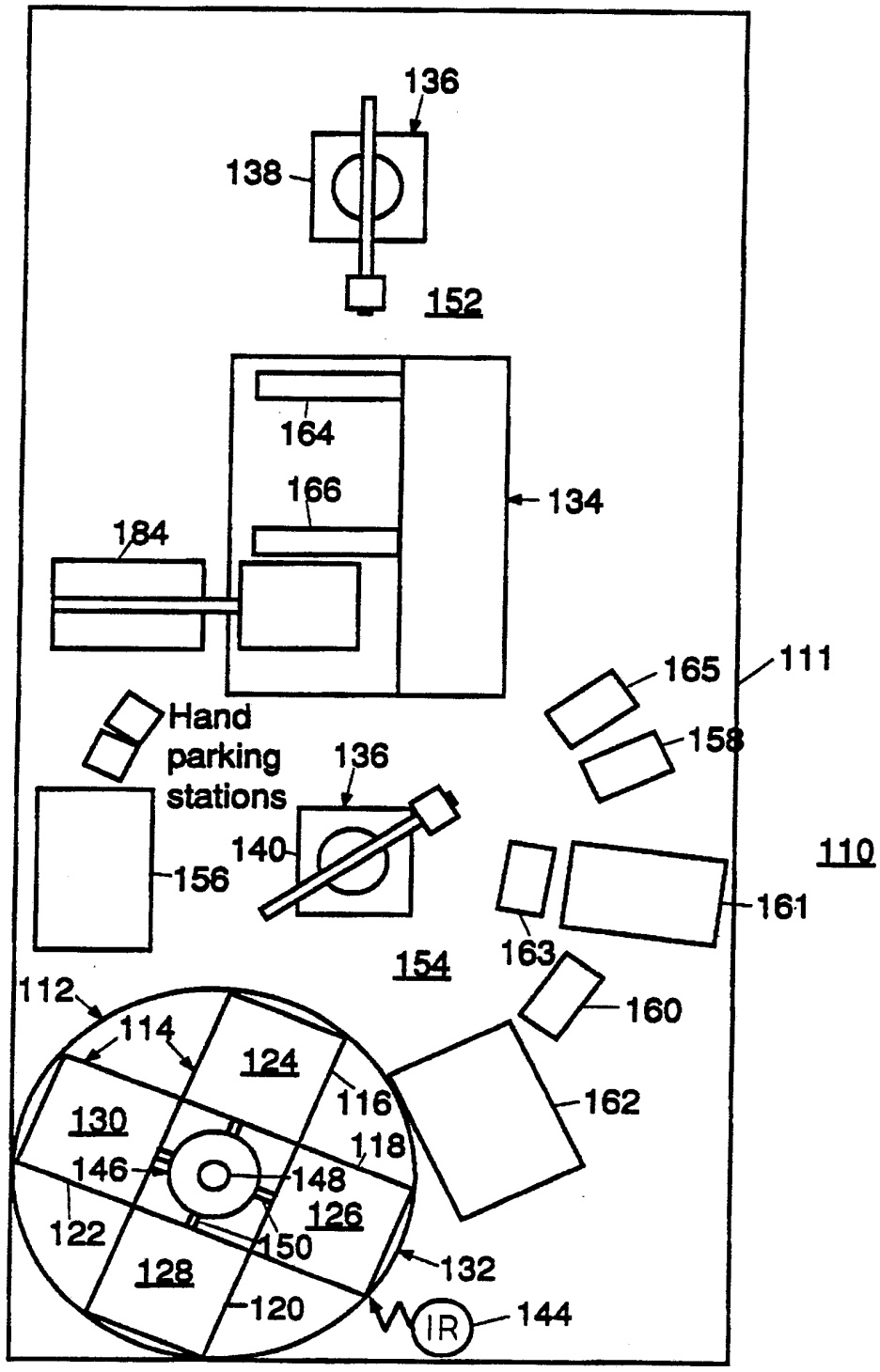
20. A laboratory plate according to claim 17 wherein said wells form a rectangular array.

21. A laboratory plate according to claim 17 formed with 96 wells arranged in an 8 x 12 rectangular array.

22. A laboratory plate according to claim 17 wherein each of said wells is formed with an open top and a bottom comprising a filter, whereby cells contained therein can be washed by a liquid entering through said top and exiting through said bottom, said filters retaining said cells in said respective wells.

23. Laboratory apparatus according to claim 4 wherein said temperature control means comprises electrical heating pads mounted on the surface of said incubation.

Fig. 1


136
138
152
164
166
134
184
165
111
158
Hand
parking
stations
136
140
156
110
161
154
163
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118
146
148
126
122
150
132
128
120
IR
144

Fig. 2

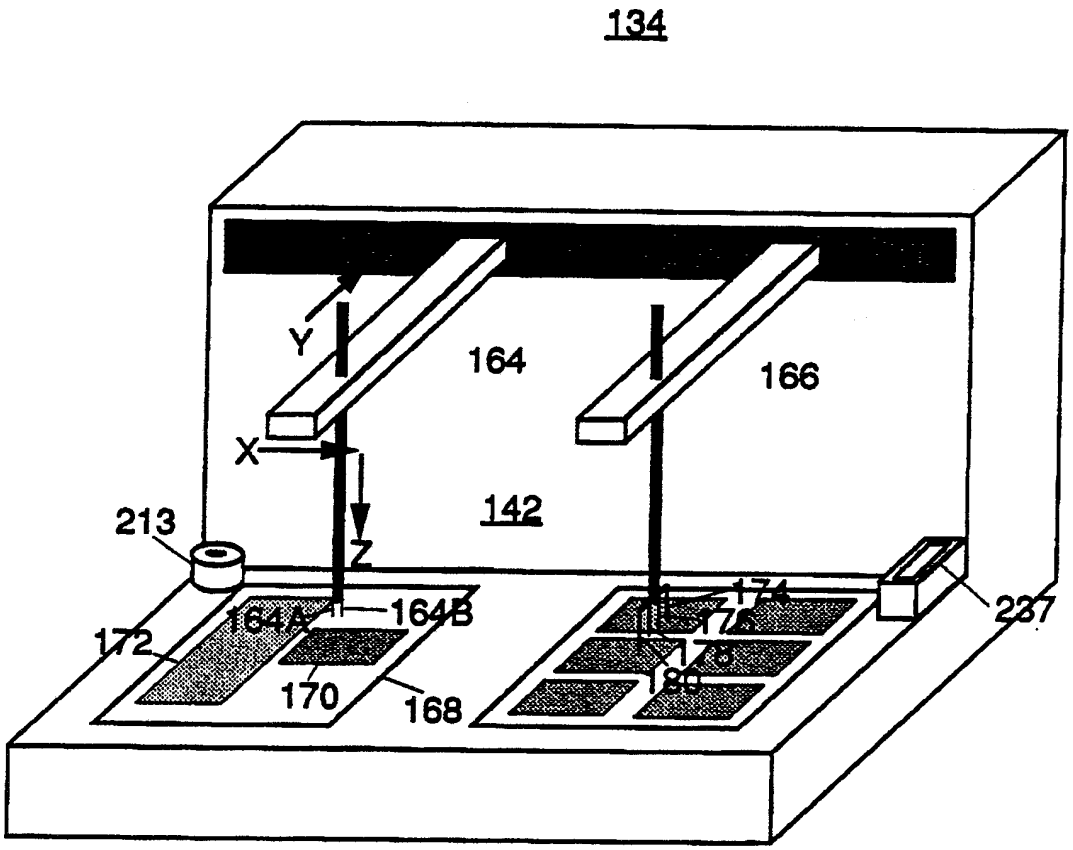

134
Y
164
166
X
142
213
Z
164A
164B
172
170
168
174
176
178
180
237

Fig. 3

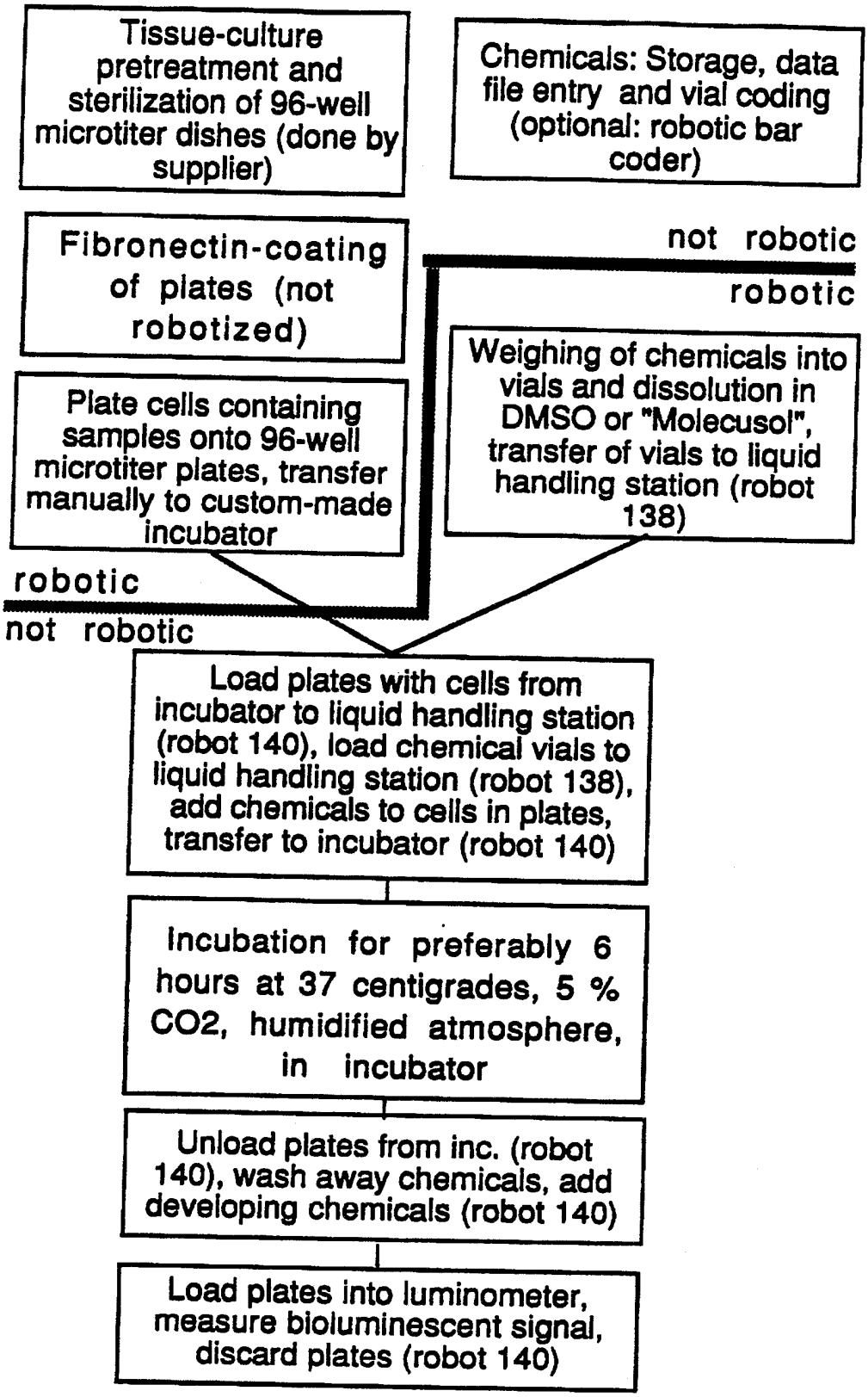

Tissue-culture pretreatment and sterilization of 96-well microtiter dishes (done by supplier)
Chemicals: Storage, data file entry and vial coding (optional: robotic bar coder)
Fibronectin-coating of plates (not robotized)
not robotic
robotic
Weighing of chemicals into vials and dissolution in DMSO or "Molecusol", transfer of vials to liquid handling station (robot 138)
Plate cells containing samples onto 96-well microtiter plates, transfer manually to custom-made incubator
robotic
not robotic
Load plates with cells from incubator to liquid handling station (robot 140), load chemical vials to liquid handling station (robot 138), add chemicals to cells in plates, transfer to incubator (robot 140)
Incubation for preferably 6 hours at 37 centigrades, 5 % CO2, humidified atmosphere, in incubator
Unload plates from inc. (robot 140), wash away chemicals, add developing chemicals (robot 140)
Load plates into luminometer, measure bioluminescent signal, discard plates (robot 140)

Fig. 4

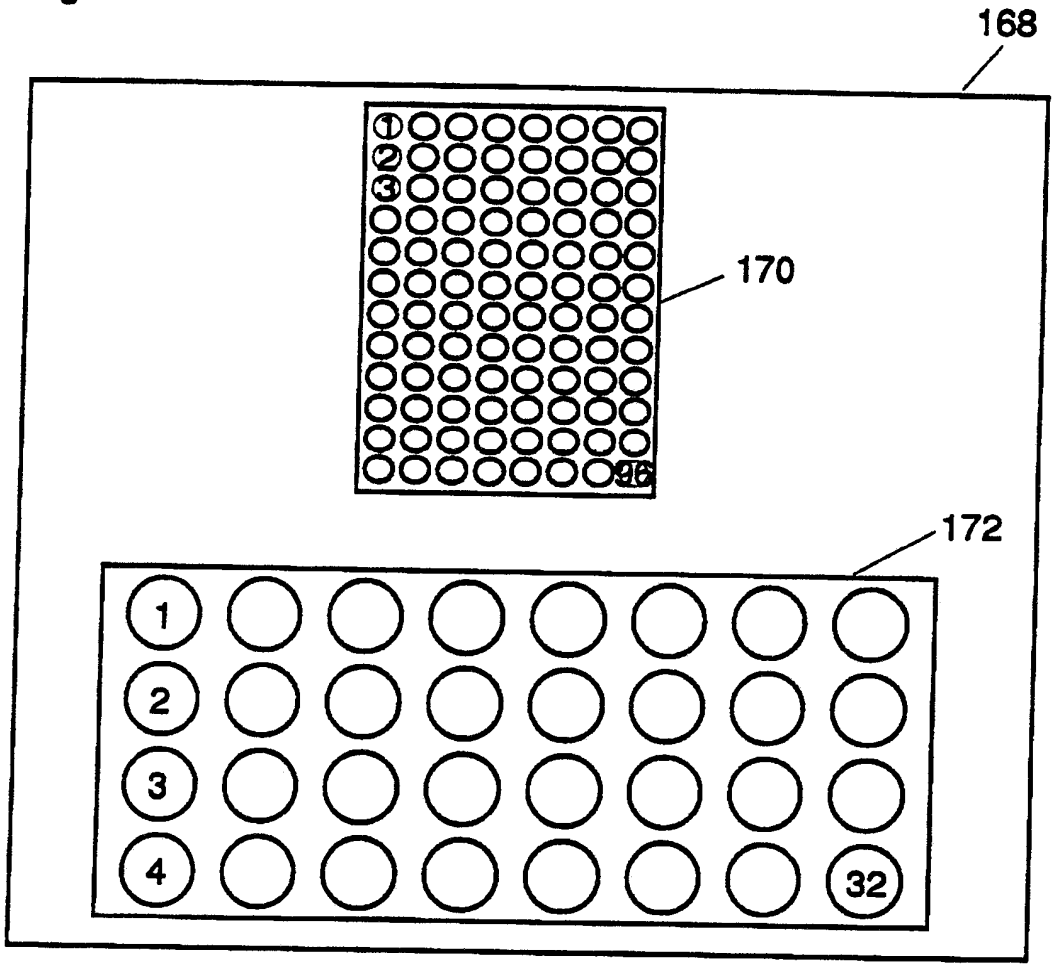


Fig. 5

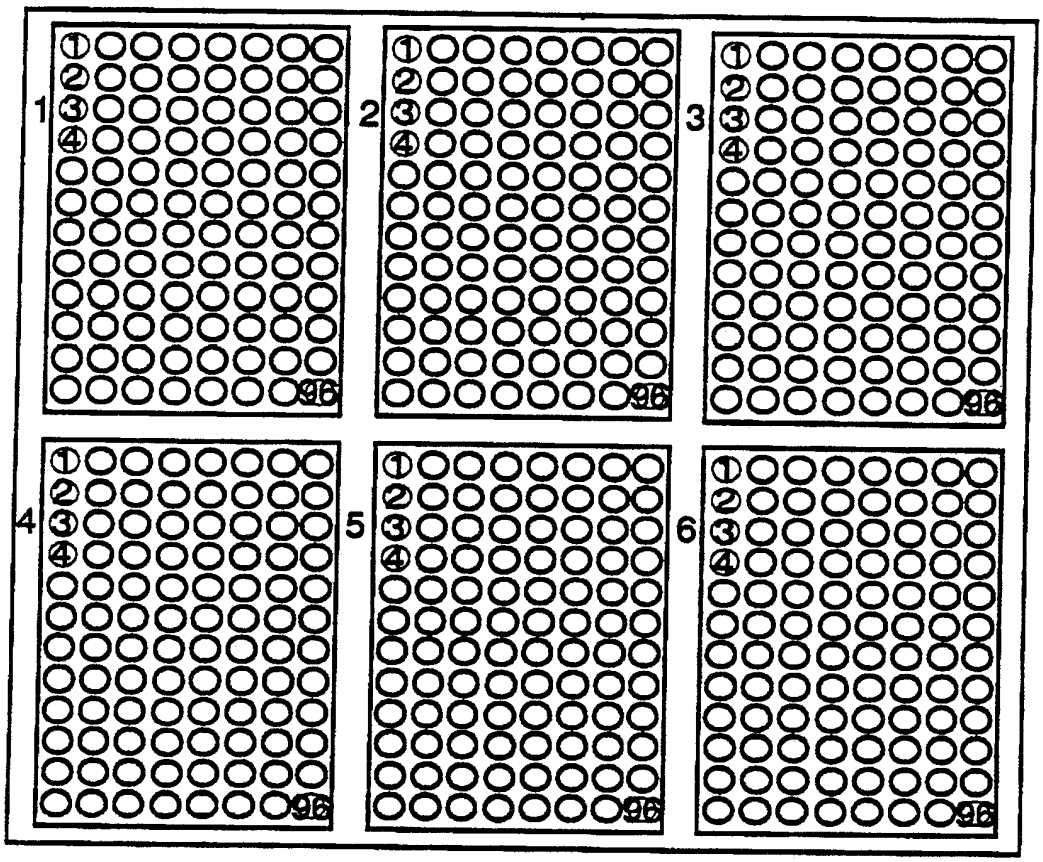
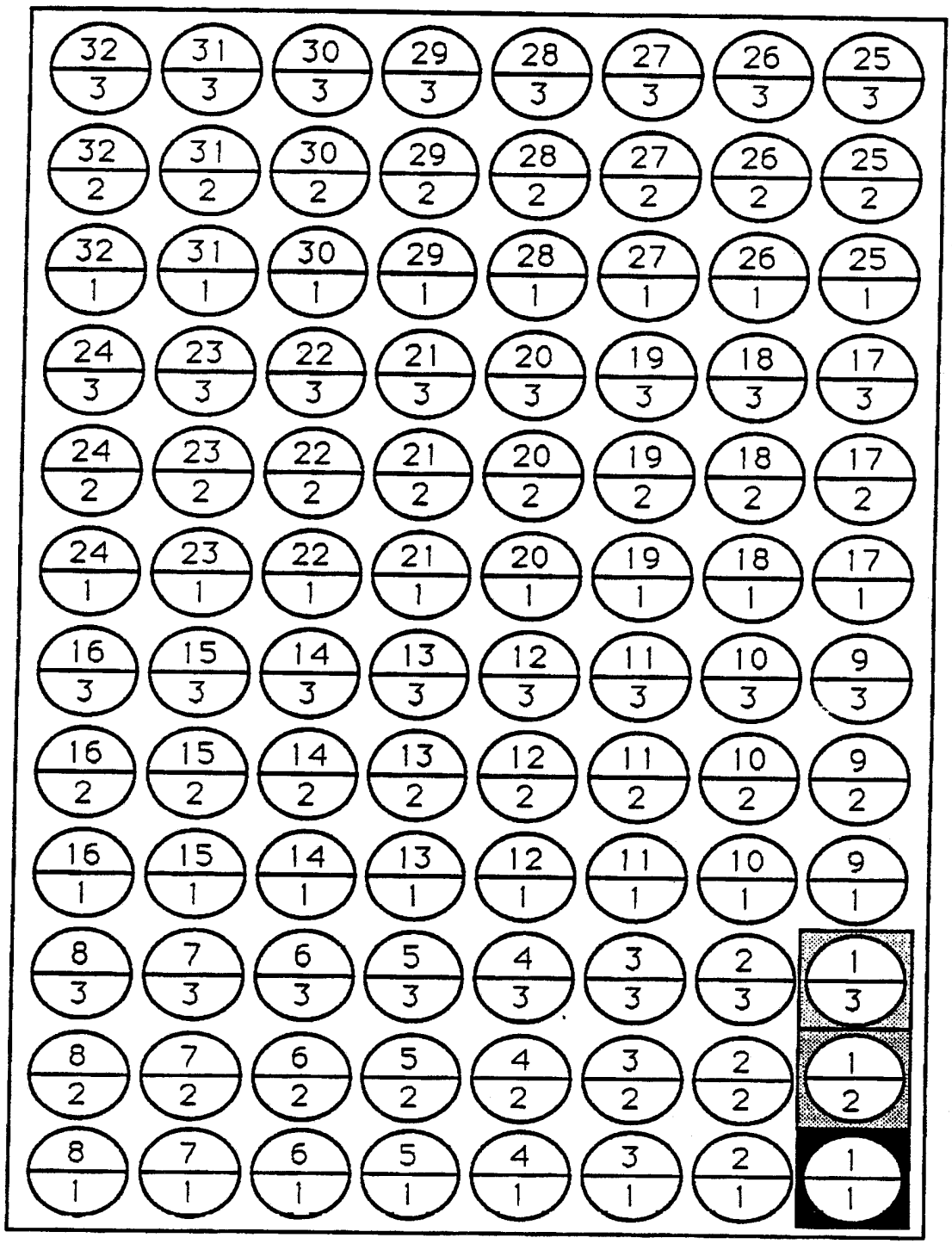


Fig. 6

170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3 3 3 3 3 3 3 3
32 31 30 29 28 27 26 25
2 2 2 2 2 2 2 2
32 31 30 29 28 27 26 25
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24 23 22 21 20 19 18 17
3 3 3 3 3 3 3 3
24 23 22 21 20 19 18 17
2 2 2 2 2 2 2 2
24 23 22 21 20 19 18 17
1 1 1 1 1 1 1 1
16 15 14 13 12 11 10 9
3 3 3 3 3 3 3 3
16 15 14 13 12 11 10 9
2 2 2 2 2 2 2 2
16 15 14 13 12 11 10 9
1 1 1 1 1 1 1 1
8 7 6 5 4 3 2 1
3 3 3 3 3 3 3 3
8 7 6 5 4 3 2 1
2 2 2 2 2 2 2 2
8 7 6 5 4 3 2 1
1 1 1 1 1 1 1 1

Fig. 7

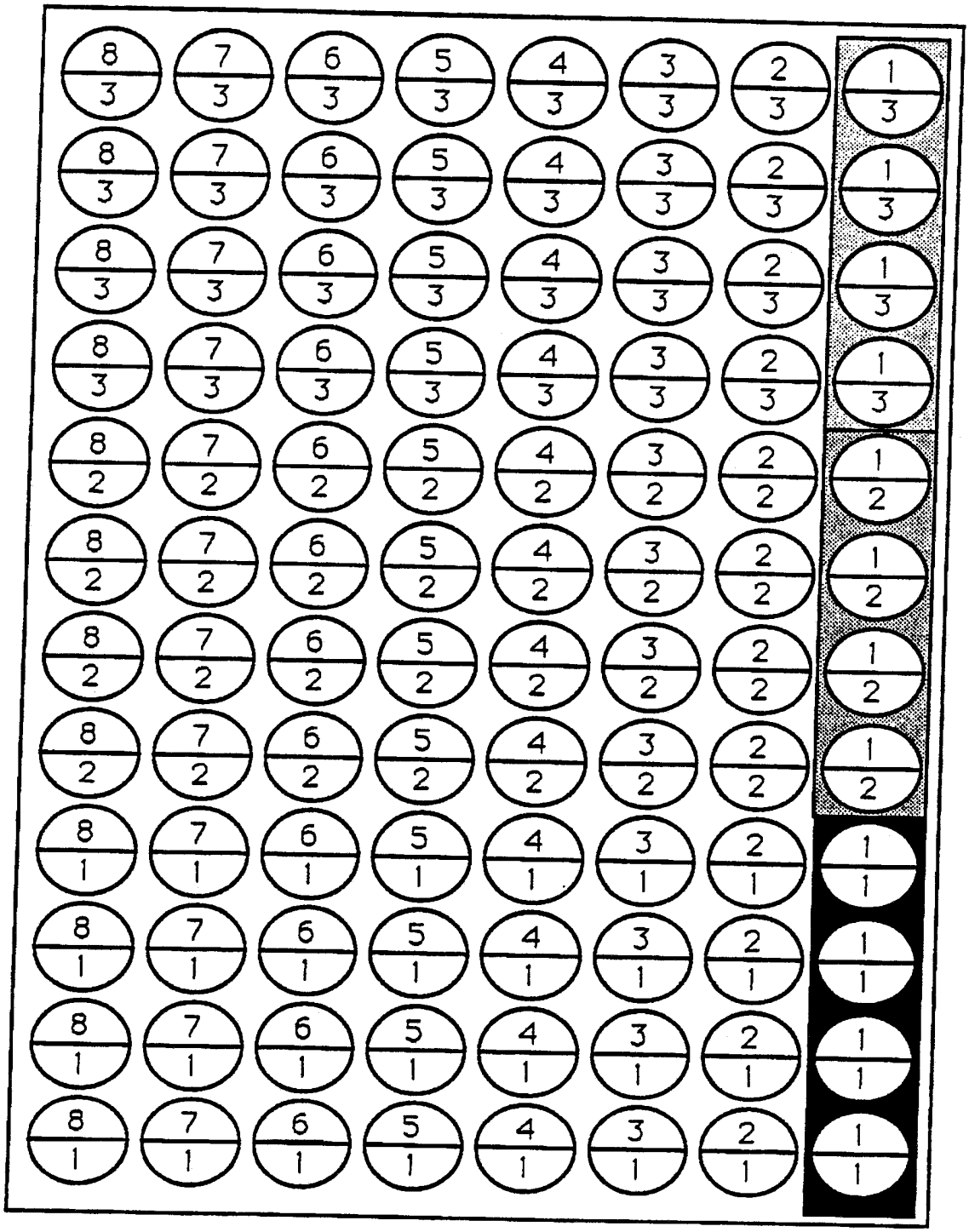
8/3 7/3 6/3 5/3 4/3 3/3 2/3 1/3
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Fig. 8

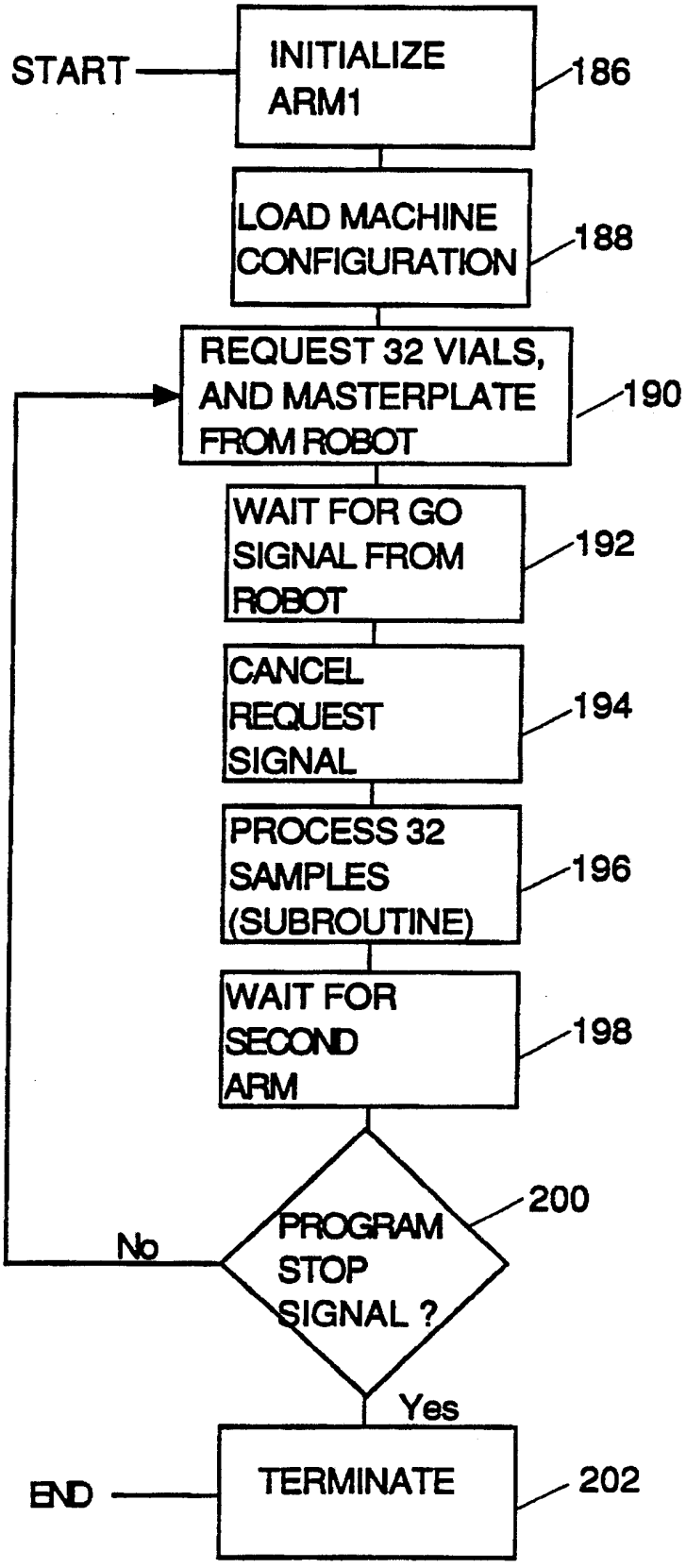

START
INITIALIZE ARM1
186
LOAD MACHINE CONFIGURATION
188
REQUEST 32 VIALS, AND MASTERPLATE FROM ROBOT
190
WAIT FOR GO SIGNAL FROM ROBOT
192
CANCEL REQUEST SIGNAL
194
PROCESS 32 SAMPLES (SUBROUTINE)
196
WAIT FOR SECOND ARM
198
PROGRAM STOP SIGNAL ?
200
No
Yes
END
TERMINATE
202

Fig. 9

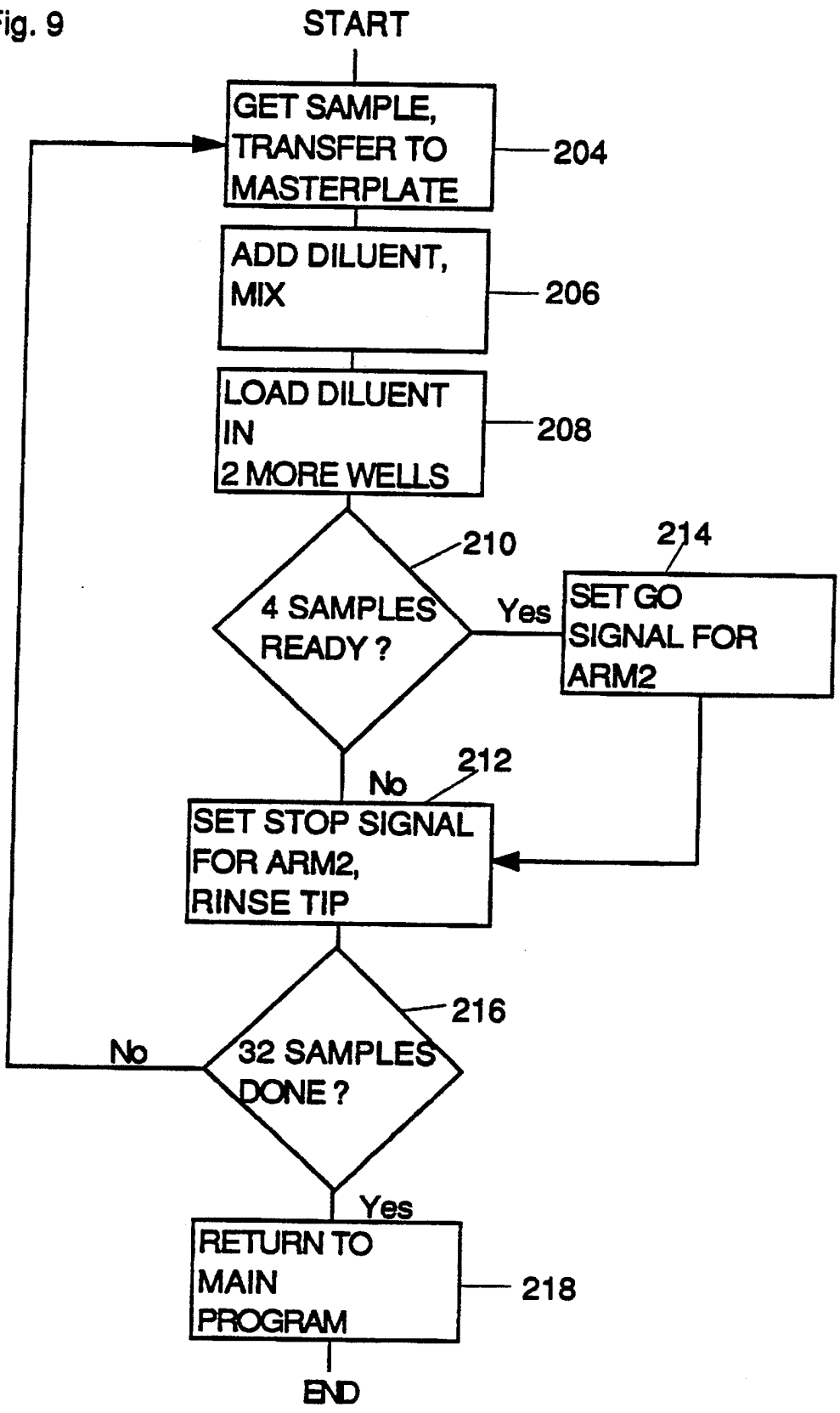

START
GET SAMPLE, TRANSFER TO MASTERPLATE
204
ADD DILUENT, MIX
206
LOAD DILUENT IN 2 MORE WELLS
208
210
4 SAMPLES READY ?
Yes
214
SET GO SIGNAL FOR ARM2
No
212
SET STOP SIGNAL FOR ARM2, RINSE TIP
216
No
32 SAMPLES DONE ?
Yes
RETURN TO MAIN PROGRAM
218
END

Fig. 10

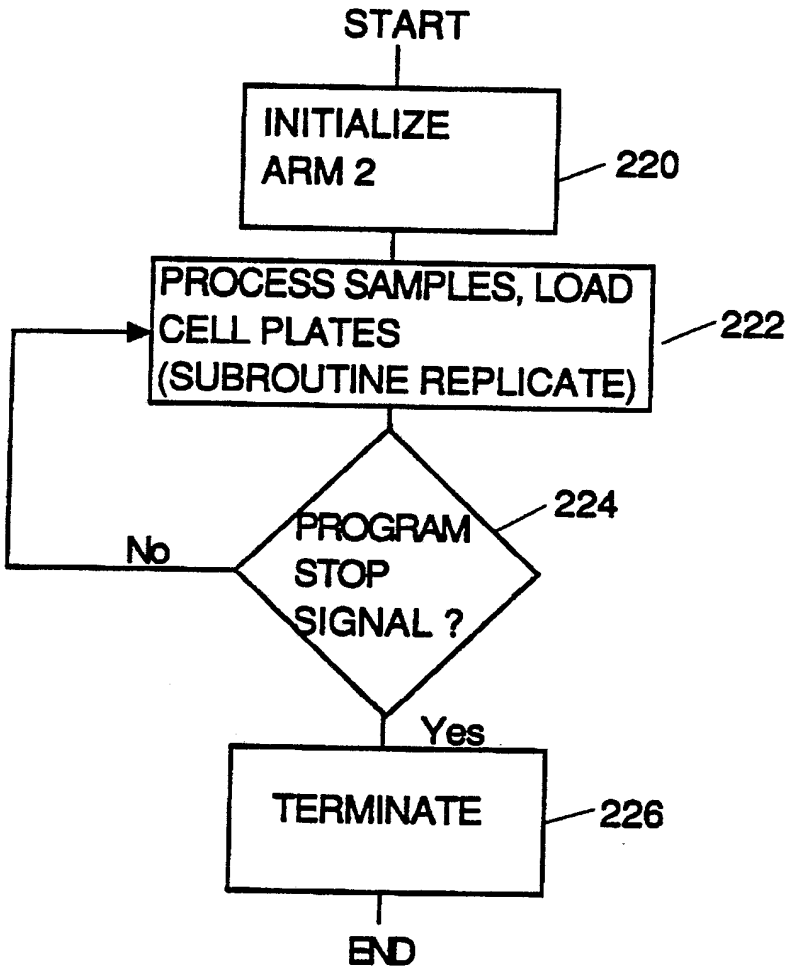
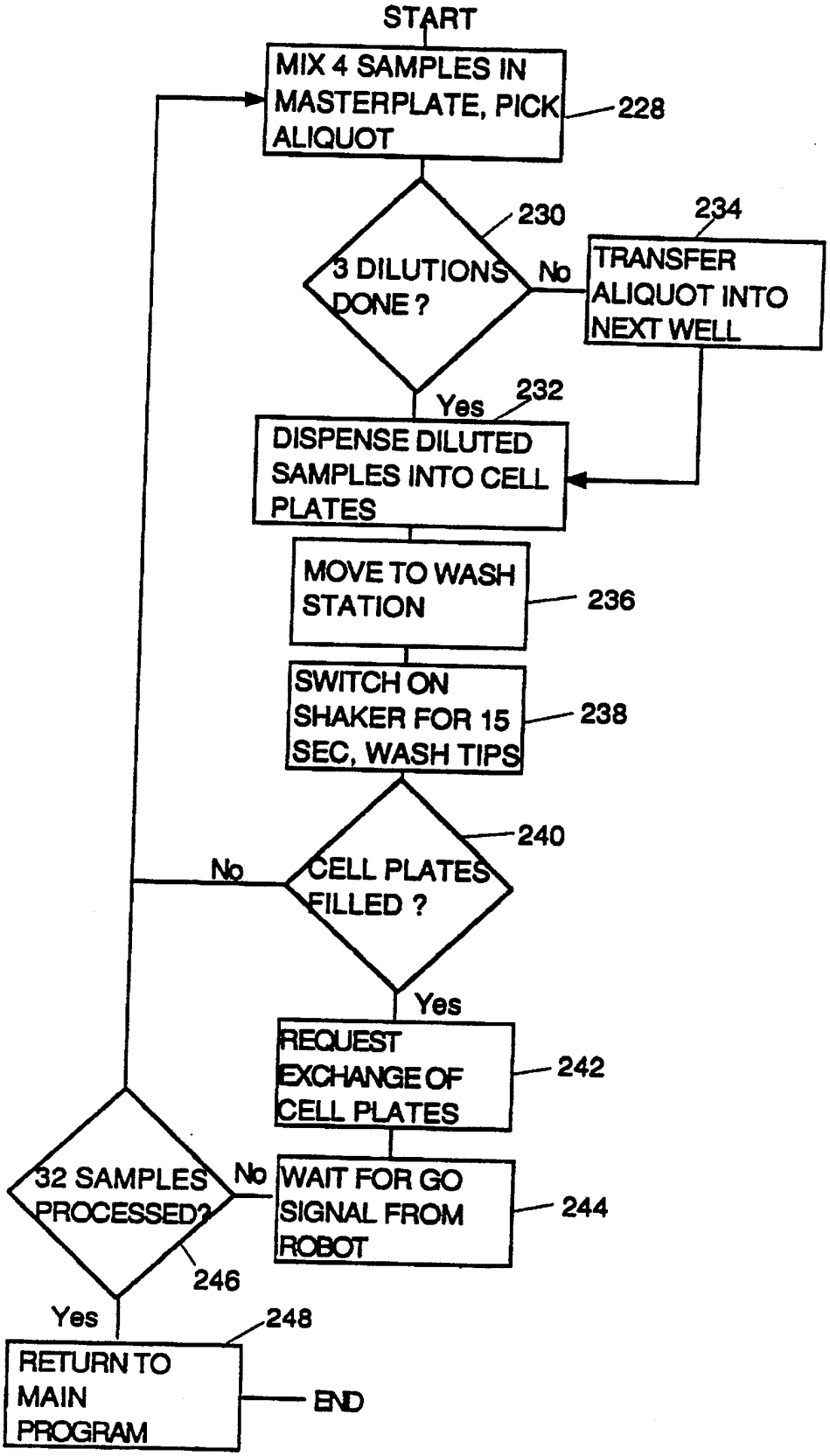

START
INITIALIZE ARM 2
220
PROCESS SAMPLES, LOAD CELL PLATES (SUBROUTINE REPLICATE)
222
PROGRAM STOP SIGNAL ?
224
No
Yes
TERMINATE
226
END

Fig. 11


START
MIX 4 SAMPLES IN MASTERPLATE, PICK ALIQUOT
228
230
3 DILUTIONS DONE ?
No
234
TRANSFER ALIQUOT INTO NEXT WELL
Yes
232
DISPENSE DILUTED SAMPLES INTO CELL PLATES
MOVE TO WASH STATION
236
SWITCH ON SHAKER FOR 15 SEC, WASH TIPS
238
240
No
CELL PLATES FILLED ?
Yes
REQUEST EXCHANGE OF CELL PLATES
242
32 SAMPLES PROCESSED?
No
WAIT FOR GO SIGNAL FROM ROBOT
244
246
Yes
248
RETURN TO MAIN PROGRAM
END

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04025

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12M 1/34;
US: 435/291

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/284-295; 422/62-67

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,720,463 (Farber et al.) 19 January 1988, see the entire document.	1-23
Y	US, A, 4,711,851 (McNamara et al.) 08 December 1987, see the entire document.	1-23
Y	US, A, 4,680,267 (Eppstein et al.) 14 July 1987, see the entire document.	1-23
Y	US, A, 4,632,808 (Yamamoto et al.) 30 December 1986, see the entire document.	1-23

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
21 August 1990	10 DEC 1990
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	T.J. Wallen

Form PCT/ISA/210 (second sheet) (May 1986)